

UNIVERSITE DE GENEVE  
Département de biologie animale

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**Contribution to reproduction  
of Amblyomma hebraeum Koch  
(Acari: Ixodidae) :  
Spermatogenesis and copulation**

THÈSE

présentée à la Faculté des Sciences de l'Université de Genève  
pour obtenir le grade de docteur ès sciences biologiques

par

Ashraf EL SAID  
du Caire

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Genève, le 26 juillet 1976

*Le Doyen:*

Jean MULLER

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## ACKNOWLEDGEMENTS

It is a great pleasure for me to express my deep gratitude to Professor André Aeschlimann, Head of the Zoology Department, University of Neuchâtel, for honouring me by including me in the general group of the tick research programme in Neuchâtel under his supervision.<sup>1</sup> I would like to convey to him my sincere thanks for his encouragement, constant help and for the task of supervising this work. Sincere thanks are also due to him for honouring me by accepting to be a member of the jury.

I am sincerely grateful to Professor Hansjörg Huggel, Director of the Laboratory of Comparative Anatomy and Physiology, University of Geneva, for furnishing me a working place in the Laboratory and for directing and contributing to this work. I would also like to thank him for his unfailing help and encouragement and for the honour extended to me in acting as a member of the jury.

My sincere thanks and appreciation are due to Dr. Zdzislaw Swiderski, who kindly undertook to technically supervise this thesis - I am deeply indebted to him for his patience, his generosity with his time, his effort and information and for having introduced me to the field of electron and freeze-etching microscopy - his direct contribution and collaboration in these fields have been invaluable.

I am sincerely grateful to Dr. P.A. Diehl, University of Neuchâtel, from whose broad scope of knowledge I have greatly benefited, for his help and encouragement throughout this work. Sincere thanks are due to him for important comments on the manuscript and invaluable advice. I am deeply grateful to him and honoured for kindly accepting to be a member of the jury.

I am profoundly grateful to Dr. Jean Wüst of the University of Geneva for his collaboration in the field of scanning.

My gratitude is hereby conveyed to Mr. Hans Bouvard and Mr. Remy Dufey of Les Barges Research Unit, Ciba-Geigy, Vouvry, for their kindness and generosity in supplying the living specimens.

I would like to thank all other members of the laboratory of Comparative Anatomy and Physiology for their co-operation, help and encouragement and for their valuable friendship, especially Mme Seif for her skillful assistance in photography.

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<sup>1</sup> These investigations were supported in part by a grant of the Swiss National Science Foundation (Request No. 3.916.72, Aeschlimann/Diehl).



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## I. INTRODUCTION

Among other parasites, ticks have become important pests in man's life. Not only do they parasitize man and his domestic animals causing annoyance, harm and loss of blood, they also constitute vectors which transmit the greatest number of various pathogenic agents.

Amblyomma hebraeum Koch, 1844, is a member of the family Ixodidae, order Acarina. This tick has been chosen for this study as it is a member of the sub-family Amblyomminae which has been largely neglected by research workers.

The availability of the material supplied by Les Barges Research Unit, Ciba-Geigy, was decisive in the choosing of this tick. Its large size, as well, facilitated its dissection and observation of the different stages of copulation.

This research work was done within the framework of the group of Professor Aeschlimann in Neuchâtel University and the cytomorphological group of Professor Huggel in Geneva University. It contributes to the phenomena of tick reproduction, mode of copulation and spermatophore structure and formation which have been poorly understood in ixodid ticks.

Observations published on the copulation process in the family Ixodidae have been few and contradictory.

This work will also contribute to the study of the tick sperm cell, which is unique in many aspects, i.e. its movement, differentiation and structure.

Since, contrary to expectations, the sperm is not transferred to the female in its mature form, further development being continued in the female genital system, it was therefore necessary to study this phenomenon in detail and to give a clear picture of the ultrastructure of the spermatid upon its arrival in the female tract, its differentiation and finally the ultrastructure of the mature spermatozoon upon completion of its development.

Moreover, we have complemented the transmission electron microscopic studies with those obtained by the freeze-etching and freeze-fracturing techniques, which have not previously been undertaken on tick sperm cells.

Scanning electron microscopical studies were also undertaken on the spermatid and the mature spermatozoon, in order to furnish a complete

external picture of their structure and shape. To our knowledge, spermatogenesis and sperm maturation in this tick have never previously been studied by electron microscopes.

## II. MATERIALS AND METHODS

### II. 1. Maintaining and feeding of specimens

Fed and unfed adult specimens of Amblyomma hebraeum were kindly provided by Les Barges Research Unit, Ciba-Geigy, Vouvry.

In the laboratory, ticks were stored prior to feeding at 80 - 90% R.H. and 20 - 25° C.

For the purpose of feeding, unfed ticks were confined in two metal capsules (2 inches wide) fixed to the backs of rabbits or in cloth earbags. These were glued to a thin sponge pad (5" by 3"). The sponge bottoms of the capsules were cut out to permit direct contact between the ticks and the host skin.

The sponge pad was then glued to a cleanly shaved area of the rabbit's back and held in position by means of adhesive tape. The ticks were then introduced in the capsules and secured by metal lids. The lids had windows of large diameters cut in them to allow aeration; these were covered with a thin piece of muslin. Removal of the capsules was easily done by cutting the growing hair of the rabbit and separating the capsule from the host.

Males were allowed to feed first for a minimum of five days. Females were then introduced with the males. This was done to facilitate the fixing of the females, since female A. hebraeum generally do not fix on hosts in the absence of males.

### II.2. Histological methods

Female ticks were dissected in Shen's saline (9g NaCl; 0.25 g KCl; 0.25g CaCl<sub>2</sub> in 1 liter of H<sub>2</sub>O).

The genital system was removed and directly fixed either in Susa, Flemming strong or Carnoy fixatives.

Tissue was dehydrated in an ethanol series and embedded in paraffin. Sections 5 - 7  $\mu$  thick were stained with Iron hematoxylin and Mallory triple stain (Pearse, 1972), mounted in Canada Balsam and examined with a Wild microscope.

## II. 3. Histochemical tests

### A. Light microscopy

Brachet's method, with the use of methyl green and pyronine, was used for the study of nucleic acids.

Feulgen method was very useful for determination of nuclear position and its migration during spermatid differentiation. This method was used on both paraffin and semi-fine sections (Kühn, 1970).

Two methods were used for the detection of alkaline phosphatase on frozen sections - (1) the modified method of Gomori for alkaline phosphatase and (2) the azo-dye method for alkaline phosphatase (Pearse, 1972).

### B. Electron microscopy

For the purpose of demonstrating glycogen, the periodic acid - thiocarbohydrazide - silver proteinate method of Thiery (1967) was used.

Acid phosphatase activity : tissue was fixed for 5 - 40 minutes with continuous agitation in cold 2% glutaraldehyde buffered to pH 7.2 with 0.1 M sodium cacodylate.

Tissue was then washed for 10 hours in 0.1 M sodium cacodylate buffer at pH 7.2, and incubated in acid phosphatase medium, according to Barka and Anderson (1962), for 20 minutes at 37° C. It was later washed for 10 minutes in 2% buffered glutaraldehyde or in 2% OsO<sub>4</sub>.

In control experiments, tissue was incubated in substrate-free medium.

## II. 4. Preparatory methods for the light microscopical observations of spermatogenesis

The ectospermatophore was removed from the genital orifice and its contents squashed in a drop of saline and examined under the microscope.

For the study of the mature spermatozoon, mated and engorged females were dissected either in physiological saline for light microscopy or in 0.1 M. cacodylate buffer (pH 7.4) for electron microscopy.

## II. 5. Transmission electron microscopy

Freshly dissected tissue was prefixed in 4% glutaraldehyde in 0.1 M. cacodylate buffer (pH 7.4) for at least 24 hours. The material was then washed in two or three changes of the same buffer for 24 hours. Post-fixation was carried out in cold 2%  $O_3O_4$  for one hour and subsequently dehydrated in successive grades of ethanol and finally two changes of propylene oxide. The tissue was left overnight in propylene oxide and Spurr mixture of 1 : 3, then 1 : 2 and finally in pure Spurr for infiltration. Embedding was subsequently made in Spurr.

Fine and semi-fine sections were cut with glass knives on a Reichert ultra microtome.

Silver-gold to grey fine sections were double-stained in uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Hitachi HS 7S electron microscope.

### Symbiont-like microorganisms

1) Intracellular - the genital organs of engorged and detached females of A. hebraeum were dissected and prepared for electron microscopy according to the methods previously described.

2) Extracellular - symbiont-like microorganisms present in the endospermatoophores were examined in squash preparations stained with Janus Green B.

## II. 6. Scanning electron microscopy

Sperm suspension was washed in saline and prefixed in 4% glutaraldehyde in 0.1 M. cacodylate buffer (pH 7.4) for a minimum of 2 hours. A drop of the sperm suspension (in glutaraldehyde) was allowed to adhere to gelatinized aluminium foil and then washed in 0.1 M. cacodylate buffer to remove the glutaraldehyde.

Post-fixative was done in 1% osmium tetroxide; rinsing was done in buffer and dehydration in an ethanol series, followed by acetone.

The aluminium foil papers were then transferred to a pressure chamber.  $\text{CO}_2$  was introduced to obtain a pressure of about 60 atmospheres. When equilibrium was established, a fine jet of  $\text{CO}_2$  was introduced to wash away acetone.

The chamber was then heated to about  $60^\circ \text{C}$ . to obtain a pressure of 85 - 90 atmospheres and to ensure that the  $\text{CO}_2$  was above the critical point. The pressure was then slowly decreased and the aluminium foil papers removed. These were then metallized with a coating of gold about  $500 \text{ \AA}$  and observed by the scanning electron microscope.

## II. 7. Freeze-etching and freeze-fracturing techniques

Tissue was dissected in saline and fixed in 4% glutaraldehyde buffered with 0.1 M. cacodylate (pH 7.4). It was then kept in 20% glycerol for 20 minutes before placing on cleaned gold disks and rapidly freezing either in Freon 12 held at  $-150^\circ \text{C}$ . or in solid nitrogen ( $-200^\circ \text{C}$ .).

The gold grids were quickly transferred to the cooled object table of the freeze-etch apparatus ( $-100^\circ \text{C}$ .).

The tissue was cut or fractured and allowed to sublime for a maximum of one minute and then coated with  $200 - 250 \text{ \AA}$  of evaporated carbon platinum.

The replicas were produced in a Balzers freeze-etch apparatus according to the technique of Moor and Mühlethaler (1963) at temperatures from  $-100^\circ \text{C}$ . to  $-190^\circ \text{C}$ .

Replicas were then immersed in 1 : 1 mixture of chloroform and ethanol for 20 minutes, next transferred to distilled  $\text{H}_2\text{O}$  where one to two drops of Javel water were added.

Replicas were rinsed in distilled water and taken up on uncoated 400-mesh grids and observed in a Hitachi HS 7S electron microscope.

### III. RESULTS

#### III. 1. Feeding

Amblyomma hebraeum, like other Ixodidae, feed once as larvae, once as nymphs and once as adults. The feeding period of each stage may last for several days.

Once detached, the female does not feed again and oviposits only once, usually over a period of 3 - 9 weeks. Upon completion of ovulation, the female dies.

Females which had not been fertilized did not succeed in feeding to repletion. Aeschlimann and Grandjean (1973) have made similar observations on Rhipicephalus evertsi. Unfertilized females either detached after feeding for some days or remained attached, but without attaining full engorgement. Once these females were fecondated, they resumed feeding until complete engorgement and consequently detached.

The size and weight increase greatly in feeding females. Fed males, on the other hand, do not exhibit great changes in size and weight.

Males of A. hebraeum, like other ixodid genera (Hyalomma Dermacentor or Haemaphysalis) cannot copulate before feeding, since sperms are found only after a blood meal of at least four days, unlike some species of ixodes which do not need a blood meal for complete fertilization.

#### III. 2. Anatomy of the female genital system (Plate I)

##### The ovary

The ovary in A. hebraeum is a single tube-like structure extending postero-laterally and acquiring a horse-shoe shape. In unfed females the ovary is thin and long and is situated in the posterior half of the body posterior to the rectal sac.

Ova develop along the entire length of the ovary except for a narrow longitudinal groove situated on the dorsal surface.

With the start of feeding, the ovary and oocytes begin a progressive development.

In mated, fully engorged females about to ovulate, the ovary greatly enlarges and occupies a large portion of the internal cavity of the female.

Developing ova distend the basement membrane and protrude above the ovary. The lateral sides of the ovary continue forward and open into the oviducts on both sides.

As the eggs reach maturation, they are ovulated into the lumen where they gather in large numbers and then pass into the lumen of the oviduct.

#### Oviducts

The oviducts are a pair of long translucent tubes extending from the lateral ends of the ovary and passing forward up the lateral sides of the vagina where they bend sharply, forming a knee-shaped loop. These regions form the ampullae.

The oviducts then continue downwards where they bend again and pass upwards to join beneath the vagina, forming a short triangular area (the uterus), which opens into the cervical portion of the vagina by a short common oviduct.

In fully engorged and mated females the oviducts greatly increase in size.

#### The ampulla

An ampulla-like region can be distinguished from the rest of the oviducts in A. hebraeum by its thick walls and its opaque white colour. Mature spermatozoa can be found in great numbers in the ampulla, either embedded in the walls or free in the lumen.

During the process of ovulation, eggs are arranged one after the other in the oviducts. In the part preceding the ampulla, the egg-shell is flexible and the eggs take an oval shape due to the pressure of the oviductal walls or irregular with flattened sides, when clusters of eggs are crammed together.

As the eggs pass through the ampulla and until they are extruded from the genital aperture, they become perfectly round and appear to be larger, firmer and more resistant to pressure. This is due to water absorption and the hardening of the egg shell (Diehl, 1970).

#### The vagina

The vagina is composed of a vestibular and a cervical portion. The vestibular vagina is short, flattened and relatively thin-walled. It leads to the exterior by the genital aperture.

#### Accessory glands

A pair of tubular accessory glands lies at the junction of both vaginas, opening postero-laterally in the vestibular vagina by a short duct.

#### The seminal receptacle

The seminal receptacle is a blind-folded sac which opens into the posterior part of the cervical vagina by a thick-walled narrow neck. The function of the seminal receptacle is to receive and store endospermatothecae. Up to five endospermatothecae can be found in the seminal receptacle of a female A. hebraeum.

### III. 3. Histology and histochemistry of the female reproductive system

#### 3. A. Ovary

In unfed adult females, the ovary is almost round in cross section. Its external surface is bound by the basement membrane. The ovarian wall is composed of epithelial cells.

As no membrane between the cells was observed, the whole layer resembles a cellular syncytium. An ovarian lumen passes throughout the whole length of the ovary.

Oocytes are carried on a stalk-like attachment of epithelial cells originating from the ovary wall, forming the pedicel.

In feeding females, the oocytes develop rapidly. Their nuclei are situated peripherally near the pedicel.

As feeding proceeds, eggs enlarge and yolk granules are deposited in the oocytes. These granules vary in size with a gradual increase, starting from the perinuclear region towards the opposite pole of the cell.

Growing eggs stretch the basement membrane and protrude above the surface. Consequently, the ovary acquires a fringed irregular surface.

The cubic cells of the pedicel continue to develop and their nuclei increase greatly in size.

The oocytes of the longitudinal groove do not continue to develop. Their nuclei remain small.

The results of staining by Feulgen and Brachet's method show that at the beginning the concentration of both nucleic acids decreases; there next appears a great amount of RNA in the cytoplasm which later decreases at the onset of yolk formation.

Eggs in the ovary of A. hebraeum, as in other ixodid ticks, do not develop all at the same time and so are oviposited over a long period.

We assume that the mature eggs separate the pedicel cells and pass through to the ovarian lumen, probably forced down by the continuous pressure of the growing eggs against the basement membrane (Lees and Beament, 1948).

### 3. B. Oviducts

Externally the oviducts are enclosed by a thin layer of connective tissue. Internally, a layer of columnar cells is present, the nuclei of which are small and oval in shape.

In feeding females, the columnar cells project into the lumen, grouped together in triangular shaped clusters. The lumen consequently acquires a star shape. After feeding, columnar cells contain granular particles. Histochemical tests indicate, at this stage, that they show evident secretory activity (Toluidine blue and Brachet's method, as well as Gomori method and azo-dye methods for alkaline phosphatase are positive).

Passing ova distend the oviduct walls, flattening their cells. A muscular layer is present externally in the connective tissue of the

oviducts and aids in the oocyte movement along the oviducts. Histologically the "uterus" resembles the oviducts in its main structure.

3. C. Ampulla

Histologically the cells of the ampulla resemble those of the oviducts, but are thicker and more glandular.

3. D. Vagina

The vestibular part is formed of a layer of endothelial cells lined with a layer of cuticle which is continuous with the body integument.

The cervical part is enclosed in a thin layer of circular muscles. This layer and a muscle band connecting the cervical region with the integument aid in the ovulation process. An endothelial layer is situated internally.

3. E. Accessory glands

In unfed females, the cells of the tubular accessory glands are small columnar with small round nuclei.

During oviposition, the cells of the glands increase greatly in size.

Large and numerous droplets are seen in the cells. The secretion of these cells is discharged in the lumen and passes through the duct which is formed of a thin layer of flattened cells bordered by a thin cuticle.

3. F. Seminal receptacle

The walls of the seminal receptacle are composed of a layer of epithelial cells with large nuclei. A thin cuticle lines the seminal receptacle.

The neck of this organ is thick-walled, due to a thick muscular band which encloses it externally.

In mated females, the seminal receptacle wall is stretched out due to the presence of the endospermatothecae. The cells acquire a secretory nature and two distinct regions can be distinguished; a vacuolated apical part and a fibrous undifferentiated basal part.

The neck region and adjacent parts of the receptacle wall show strong positive reaction to alkaline phosphatase by Gomori's method.

### III. 4. Copulation

The Metastriata group, of which Amblyomma hebraeum is a member, is known to copulate on its hosts. Adults are commonly attached in couples on the host for feeding and copulation.

A. hebraeum females search for and attach near the males, and not vice versa. Moreover, females do not fix on the host in the absence of males.

During the course of this study, it was noticed that the female tick copulates from 1 to 5 times (observations under laboratory conditions).

Upon dissection of engorged females, sperm were usually found in the ampulla oviduct and very rarely in the ovaries. However, on two separate occasions, we noticed that two engorged females failed to oviposit 35 and 37 days after dropping in contrast to 8 - 20 days for normally copulated ones. Upon dissection of these females, no sperm were found in any part of their genital systems, but debris and remnants of what might have been endospermatophores were found in the seminal receptacles. If copulation had taken place, it was probably incomplete or abnormal. Mating in this case probably did not result in the transfer of sperm cells to the female since none were found in the endospermatophores. Sperm cells may therefore play a role in the final stage of egg development. Only normally copulated females succeeded in oviposition.

Males have not been able to copulate before feeding, since spermatids are found only after a blood meal of at least five days.

Female A. hebraeum usually prefer to mate only when they are partly replete; however, some females with no significant change in their size were dissected and found mated.

Unmated partly-replete females either detached or remained fixed for long periods without attaining full repletion. When mating finally took place, they quickly fed to complete repletion and detached.

#### Process of copulation (Plate II)

In ticks the process of insemination is accomplished by the male's injection of sperm and seminal fluid in a spermatophore which is transferred to the female where its contents evaginate in her genital system.

To observe the process of copulation, potent males were placed on the ventral surface of immobilized receptive females.

The male starts to creep about her and advances so as to bring his body into line with hers and places his ventral surface in close proximity to hers. The male then clasps the female with his legs which he folds with those of the female. He next bends his mouth parts at right angles with his body and directs them vertical to the genital orifice. He maintains this position throughout the whole process of copulation.

The male introduces the cheliceral digits in the female genital orifice, and on close observation these can be seen protruding from their transparent shafts. The palps and hypostome are not included in the female genital orifice and remain outside (Plate II, Fig. 1).

The genital orifice of the female is small in size and consequently cannot make room for the chelicerae, hypostome and palps all together. Furthermore, the orifice is flanked on both sides by two chitinous chips. These prevent the further expansion of the orifice. The genital orifice of the female becomes deeply depressed, as well as the region posterior to it, forming a shallow groove.

Saliva starts oozing from the male mouth parts; he then lifts his body slightly and an ectospermatophore begins to issue from the male genital orifice (Plate II, Fig. 2). This bulb is attached to the male genital orifice by a short narrow neck. The period between the onset of copulation and the apparition of the ectospermatophore takes about 20 - 25 minutes.

At first, the empty ectospermatophore is colourless and transparent; a few seconds later sperm, seminal fluid and granules are injected into it, changing its colour to milky white. Finally, it is stoppered with the endospermatophore and its formation is complete. The process of spermatophore formation does not last more than 1 to 2 minutes (Plate II, Fig. 3). The various components of the ectospermatophore are secreted by the male accessory glands (Tatchell, 1962).

Upon the completion of the ectospermatophore, its neck detaches from the male genital orifice and turns towards the female genital orifice (Plate II, Fig. 4). The male mouth parts, which never leave the female

genital orifice, help in implanting the neck of the spermatophore into the female genital orifice (Plate II, Fig. 5).

The male mouth parts do not leave the genital orifice of the female at any time nor do they transfer the ectospermatophore to the female genital orifice. The neck of the ectospermatophore reaches the genital orifice of its own accord.

When copulation is terminated, the male walks off, leaving the empty ectospermatophore still attached to the female genital orifice. Upon examination of the latter, a part of the endospermatophore neck is usually seen to be attached to it. The whole process of copulation takes about 25 - 30 minutes.

### III. 5. Evagination of the endospermatophore (Plate III)

The evagination process has taken place in the laboratory in saline or tap water and is completely autogenous. The male mouth parts do not cut the ectospermatophore tip to allow evagination to occur nor does the male pump out its contents.

At first, a finger-like process events outwards, forming the endospermatophore neck (Plate III, Fig. 1). Finally, a bulb-like endospermatophore appears at its tip (Plate III, Figs. 2 and 3).

Upon completion of the evagination process, the neck of the endospermatophore then breaks at a point near the endospermatophore lobe. The evacuation of the ectospermatophore contents is believed to be triggered by the pressure of CO<sub>2</sub> gas formed in the ectospermatophore (Feldman-Muhsam et al, 1973).

The ectospermatophore is mainly composed of three layers :

- a thick sticky envelope situated externally;
- a non-cellular thick layer which gives the ectospermatophore its bulb shape;
- a thin membrane, located internally, lines the ectospermatophore enclosing its contents.

### III. 6. Endospermatophore

The number of endospermatophores found in the seminal receptacle of one female correlates with the number of times she has mated.

Endospermatophores of A. hebraeum are one-lobed and each bears two tubes, one long and thick, the other short and thin.

### III. 7. Symbiont-like microorganisms

During the course of this study, we have noticed symbiont-like microorganisms in association with the sperm cells of A. hebraeum. These yeast-like microorganisms are extracellular symbionts, produced by the male and introduced in the genital system of the female through copulation (Feldman-Muhsam and Havivi, 1963).

These microorganisms are frequently in large agglomerations and are in the form of compact sperules and morulae. They do not change their spherical shape (Feldman-Muhsam and Filshie, 1974) as do other such symbiotes found in Ornithodoros tholozani, which become spindle-shaped, nor do they attach to the sperm cells.

Another kind of Rickettsia-like microorganism is present in the tissue of some organs of A. hebraeum. As the tick specimens with which we worked were infected with Rickettsia cooneri (fièvre boutonneuse), it is not clear whether these microorganisms are the disease agents or naturally occurring Rickettsia-like microorganisms previously reported by many authors (Aeschlimann and Hecker, 1970, and Reinhard et al, 1972), as being present in the tissue of many organs of both male and female ticks.

### III. 8. Ultrastructural aspects of spermatogenesis

Spermatogenesis in Argasid and Ixodid ticks is basically similar in its main stages. In this study, we have reviewed the general pattern of this process in the male and the main stages that lead to the formation of the spermatid stage finally transferred to the female which we have studied in detail, together with the mature spermatozoon.

#### III. 8.A. General pattern of spermatogenesis (Plate IV)

Spermatogonia multiply by several mitotic divisions and finally develop into primary spermatocytes (Plate IV). These cells undergo extensive growth, increasing the cytoplasm contents and size of nucleus and nucleolus. Golgi elements and endoplasmic reticulum can be observed in the cytoplasm.

Maturation of the primary spermatocytes initiates in the posterior most part of the testis and proceeds in a wave-like fashion anteriorly (Oliver, 1974). The primary spermatocytes divide by meiosis, resulting in secondary spermatocytes.

The spermatids resulting from the second maturation division are spherical cells characterized by the formation of a thin wall of sub-surface cisternae. We have observed a gradual increase in the size of vesicles towards the sub-surface cisternae. These cisternae may therefore be derivatives of Golgi vesicles, as demonstrated by Reger (1974) in Dermacentor andersoni.

As the spermatid develops, the nucleus migrates to one pole and assumes an eccentric position and the Golgi elements move towards the nuclear pole. The nucleus becomes compact, decreases in size and assumes an elongate shape.

The cisternae at the nuclear pole retreat while those of the opposite end develop rapidly and elongate. The distance between neighbouring cisternae increases which may be due to a process of cisternal fusion demonstrated by Reger (1974) in D. andersoni. This may therefore result in the decrease in the number of cisternae and the appearance of a cisternal cavity in the posterior half of the cell reported by other investigators (Casteel, 1971; Sharma, 1944; Khalil, 1969 and Reger, 1974).

The cell at this stage begins to elongate in all species studied (Casteel, 1917; Tuzet and Millot, 1937; Sokolov, 1958; Wagner-Jevseenko, 1958; Breucker and Horstmann, 1972, and others). The continuous fusion of cisternae finally results in the formation of one large cisternum enclosing a large cavity in D. andersoni (Reger, 1974).

A process universal among tick species studied in the next stage is the extension of a finger-like process from the mitochondrial end of the cytoplasm into the cisternal cavity (Plate IV, Fig. 7). This structure is virtually the tip of the inner cord.

At this stage, Reger (1962) observed in Amblyomma dissimili numerous small bulbous expansions at the tip of the inner cord.

Meanwhile, the elongated nucleus migrates in the outer sheath to the opposite end of the tubular cell (Plate IV, Fig. 8), and is finally situated at the anterior end of the cell underlying the cell membrane

(Plate IV, Fig. 9).

The advancing inner cord eventually reaches the opposite end. The end product of this transformation is an elongated cell composed of two tubes, the outer sheath and the inner cord, the former surrounding the latter (Plate IV, Fig. 9).

The spermatid, in this form, represents the final developmental stage reached in the male and is stored in the vasa deferentia until copulation takes place.

### III. 8.B(a) Scanning electron microscopy

Spermatids of A. hebraeum are large cells (60 - 100  $\mu$  long) and tubular in shape. The anterior end is tapering, while the posterior part is truncate.

At this stage, the nucleus is situated at the anterior pointed end of the sperm cell and runs down to about half its length. Its location can easily be spotted as it bulges out over the spermatid surface.

The external surface of the spermatid is smooth, except for a well defined rim at the anterior end. This rim defines a cap or operculum (Borut and Feldman-Muhsam, 1976) from the rest of the sperm body. The significance of this finding will be discussed later.

### III. 8.B(b) Comparative freeze-etch replicas and thin section studies

The main body of the spermatid is composed of two long tubes, one inside the other, the outer sheath and the inner cord, the former surrounding the latter.

The lining of the outer sheath and the surface of the inner cord is covered by longitudinal cellular processes joined together at the truncate end of the sperm cell.

The electron dense nucleus is located in the outer sheath of the spermatid and lies externally over the layer of cellular processes just beneath the plasma membrane of the outer sheath.

The nuclear membrane persists and does not disappear at any stage. Sections through the anterior end of the spermatid show a thick double-walled membrane surrounding the outer sheath. This structure is probably the flat disc-shaped acrosome reported by Reger (1963) in Amblyomma dissimili.

At the anterior tip of the inner cord, a semi-spherical structure is located. This is a complex structure which, when exposed in the mature spermatozoon, may have a tactile function (observations of spermatozoon movement).

The "tactile" semisphere bears numerous bulbous expansions, each attached to the surface by a thin stalk-like connection.

A freeze-etch replica reveals a peculiar pore-like depression on the surface of each bulbous expansion. These pores are peripherally situated on each expansion and assume a parallel arrangement on the posterior-most rows of the bulbous expansions. A gradual transformation in the size and shape of the bulbous expansions can be observed on freeze-etch replicas of this region.

A short ring of motile processes extends over the last row of expansions and is in constant motion in the mature spermatozoon (Casteel, 1917). These processes are a row of extended bulbous expansions which are attached to the semi-spherical structure by short stalks. Longitudinal cellular processes run down the length of the inner cord, covering its entire surface. At the posterior tip of the cord, these cellular processes fold externally upon themselves and run up the outer membrane to the pointed end of the outer sheath.

Each longitudinal cellular process is probably attached to the sperm proper by a thin connecting tube, as demonstrated by Breucker and Horstmann (1968) in Ornithodoros moubata.

In cross-section, the cellular processes are seen to be arranged in two concentric circles. Each is pear-shaped in cross-section and is composed of two sub-units of varying electron density. The first (type I) is homogenous and of relatively low electron density. The second (type II) is crescent-shaped and darkly staining; it limits type I at the broad end of the pear-shaped cellular process. A terminating cellular process (type III) is characterized by its dwarfed size and two filaments present in it.

Whereas the tip of the inner cord is void of mitochondria, the region just posterior to it is packed with relatively great numbers of mitochondria, forming a plug-like region. Elsewhere in the spermatid body mitochondria can also be seen, but randomly dispersed and few in number.

On close examination of the mitochondria, many are found to contain electron dense granules.

The cytoplasm in the anterior part of the inner cord is granular and moderately dense. That of the middle or posterior region of the inner cord is of a fibrillar-granular nature. The granules may represent the fibres in cross-section.

The fibrillar-granular material of the cytoplasm at the lower end of the cell accumulates as dense clusters lining the inner surface of the inner tube and bordering the outer sheath.

In the cortical region, there are also numerous dense fibres which on some longitudinal sections appear as running the length of the spermatid.

A longitudinal fold of the inner cord is seen in the thick anterior part of the spermatid.

### III. 8.B(c) Elongation process

The final development of the spermatozoon is completed in the endospermatophores contained in the seminal receptacle within one hour of copulation. This development involves the unusual doubling of the spermatid size forming the mature spermatozoon.

The spermatid, upon its transfer to the female, is closed at both anterior and posterior ends. Shortly afterwards, a cap is detached from the anterior end and the elongation process is permitted to take place. Scanning micrograph of the external surface of the spermatid reveals the rim of this cap. Consequently the semisphere, followed by the inner cord, passes through the resulting opening. Meanwhile, the outer sheath, carrying the nucleus, slips backwards and turns inside out at the truncate end of the elongating spermatid.

Once freed from the outer sheath, the apex of the inner cord folds out and swells, forming the eventual club-shaped anterior part of the spermatozoon.

This extra thickness is due to the outward expansion of the longitudinal fold, seen in cross-section of immature spermatids.

The evagination of the inner cord and the invagination of the outer sheath take place simultaneously and continue until finally

both outer and inner tubes are merged. As a result, a single club-shaped structure (about 120 - 200  $\mu$  long), double the length of the original spermatid, is formed.

### III. 8.C. The mature spermatozoon

The mature spermatozoon later leaves the endospermatophores, probably through the thick tube, and migrates up the oviduct and ampulla. As the internal structure of the spermatozoon is similar to the inner cord of the spermatid, only the differences observed in both stages will be mentioned.

#### III. 8.C.(a) Scanning Electron microscopy

The final external features of the mature spermatozoon are characterized by a thick, club-shaped anterior end carrying at its tip the "tactile" semisphere.

The surface of this sphere is covered by numerous small expansions. In the mature sperm, this region does not change greatly from that in the tip of the inner cord of the spermatid.

The external surface of the mature spermatozoon, after being freed of the outer sheath, appears ridged, due to the presence of the cellular processes.

#### III. 8.C.(b) Comparative freeze-etch replicas and thin-section studies

In cross-section, the mature spermatozoon differs mainly from the spermatid in having just one circle of cellular processes in contrast to two concentric ones in the spermatid.

The electron dense nucleus is finally located internally at the posterior half of the tail-like region closely adjacent to the invagination membrane.

As a result of the invagination of the former outer sheath during spermatid elongation, the "acrosome" is finally located in the nucleate tail-like end of the sperm surrounding the acrosomal canal.

The cellular processes run more or less parallel to each other. Each process continues for a certain distance along the external surface, after which it terminates and is usually followed by another. Short stalks are probably connecting these processes with the sperm cell body.

In some cases, a terminated cellular process is not succeeded by another and the flanking cellular processes on both sides draw nearer together until they ultimately run parallel. In some regions these processes take a somewhat wavy course.

The membrane-associated particles found on the membrane underlining the cellular processes show their peculiar differentiation as revealed by freeze-etching technique. This is in the regular arrangement of batches of these particles in numerous parallel strands.

Mitochondria are not regularly arranged in rows as in Ornithodoros papillipes (Sokolov, 1958) and O. moubata (Breucker and Horstmann, 1968), but are simply grouped together in the advancing sperm end.

Glycogen representing individual  $\beta$ -particles is present in the mature sperm cell.

The dense clusters observed in the inner cord of the spermatid now appear in the cortical region of the cytoplasm usually underlying the spermatozoon plasma membrane.

In the mature spermatozoon, large granules appear at the periphery and along the thin part of the sperm cell. These granules are extra-cellular and are located between the cellular processes and the plasma membrane which separates them from the cortical cytoplasm.

In older spermatozoa found in the oviducts and more often in the ampulla, these granules become more dense and appear to have a granular matrix.

The external surface of the peripheral granules, as revealed by freeze-etching, is smooth with a very small number of particles. Internally, the particles exhibit a regular hexagonal pattern which has not been observed, so far, on any other membrane in these sperm cells.

The rod-shaped nucleus in the mature spermatozoon usually runs adjacent to the invagination membrane.

The nuclear envelope is always present in the mature spermatozoon.

Since fertilization in ticks has rarely been observed, the structure and the actual role of the "acrosome" in penetration of the egg has never been described. In A. dissimili, Reger (1963) described an "acrosome" consisting of a flat, disc-shaped vesicle situated together with the nucleus at one end of the spermatozoon. The origin of this

vesicle appears to arise as a fusion of dictyosomal vesicles.

In A. hebraeum a similar structure is observed in young spermatozoa found in the endospermatophores within the seminal receptacles.

In the spermatid stage, we have observed this structure at the anterior end lining the cellular membrane. And as a result of the invagination of the outer sheath, this structure, together with the nucleus, is finally located in the tail-like end of the sperm.

As the spermatozoa age in the ampulla region, this structure develops and its association with the nucleus can be easily observed (Plate VI).

The acrosomal plate (Plate VI) is characterized as a moderately dense plate lining part of the invagination membrane facing the nucleus. This plate runs for a short distance below and above its point of attachment with the nucleus.

The base of the nucleus is crescent-shaped and adheres the nucleus to the acrosomal plate. A long thin moderately dense rod is embedded in the nucleus and runs up inside it for a certain distance. The apparent function of this rod is to further strengthen the nucleo-acrosomal liaison by fixing the acrosomal canal to the base of the nucleus. The other end of the nucleus is free.

The invagination membrane lines the acrosomal canal that has come to exist as a result of the invagination of the outer sheath during the elongation process.

### III. 8.C. (c) Motility

The sperm was seen to move in a gliding manner with the club-shaped part leading, followed by the thin tail-like end. This movement is also characteristic of other tick sperms (Casteel, 1917; Rothschild, 1961; Reger, 1974).

Occasional rotations of the cell have also been noticed. Due to the flexibility of the cell surface, the tail-like part can sometimes be seen bending on either side.

On close observation, the bulbous expansions on the semisphere surface are seen to be in continuous motion, as well as the fringe of

motile processes surrounding it. The semisphere may have a tactile function.

A peculiar rolling-up and extending outwards motion of the "tactile" semisphere is observed in living sperms.

A longitudinal depression in the club-shaped part accompanies the extending outwards motion. This depression disappears when the semisphere is rolled up.

### III. 8.D. Fertilization

After the completion of feeding, the endospermatophore - probably through one of its two tubes - liberates the sperm cells. The liberated sperm cells move up the oviducts and are found dispersed along the length of the oviducts, but rarely in the ovaries. The greatest number of sperm has been found in the ampulla-like region.

At the ampulla region, the passing ova are surrounded by the numerous sperm cells. The fact that the important development of the acrosome occurs when the sperms are present in the ampulla region and just before the onset of ovulation increases the possibility that fertilization occurs in this region.

It is unlikely that the whole sperm cell enters the ovum for its fertilization. As the nucleus and "acrosome" are situated in its thin tail-like region, it is probable that only this region is involved in the process of fertilization.

However, the actual process of fertilization, despite repeated attempts, remains unobserved by us.

### III. 8.E. Phagocytosis

The great number of mature spermatozoa that have been released from the endospermatophores migrate to the oviducts and ampulla. In these regions, the sperm cells break down by the action of mainly two processes.

The first involves the enzymatic action of the ampulla wall cells on the spermatozoa found intracellularly. A test for acid phosphatase in the ampulla wall indicated positive reaction in autophagic vacuoles surrounding the sperm.

The second process is observed in aging sperm cells. At this stage, lysosome-like structures appear in the cytoplasm of the spermatozoa which probably perform an autophagic function involving the intracellular digestion of cellular components.

The dying or disintegrating sperm cell exhibits certain structural deformations which could be attributed to cell aging.

#### IV. DISCUSSION AND CONCLUSIONS

In A. hebraeum, the ovary and oviducts are longer and thinner as compared to those of members of Argasidae.

The ampulla region in Ixodid females has not previously been reported. Balashov (1964) fails to mention it; Oliver (1974) concludes that there are no specialized ampulla in Ixodid females.

Casual observation could overlook this structure, since in unfed females of A. hebraeum it is only slightly thicker than the oviducts. However, in fully engorged females just before ovulation, the ampulla can easily be distinguished as a knee-shaped thickening of the oviduct, the limits of which are also easily distinguished.

Great numbers of sperm have also been found in this region as in the case of the ampullae of other species (Balashov, 1964). The phagocytic function of this region is very prominent in A. hebraeum; large quantities of sperm are apparently phagocytized by the cell walls. In conclusion, the presence of the ampulla region in this tick (a member of Ixodidae) must be stressed.

The "uterus" in this tick does not receive the endospermatophores. The reception of the endospermatophores is performed by the sac-like seminal receptacle. This organ is generally present in all members of ixodidae, with the exception of Ixodes ricinus (Roshdy, 1969). The function of this organ is carried out by the common oviduct in I. ricinus (Graf, 1976) and by the uterus in Argasidae.

Copulation and insemination in ticks have been described by many authors (Feldman-Muhsam, 1963, 1971; Ammah-Attoh, 1965, 1966; Balashov, 1968; and Oliver et al, 1974). Some differences of opinion exist between authors on the mode of copulation of ticks belonging to

the different families and groups. Arthur (1962) erroneously believes that the mating behaviour is similar in Argasidae and Ixodidae.

In all tick species studied, the male transfers the seminal fluid and sperm to the female by means of the ectospermatophores which are secreted by the different male accessory sex glands of the genital system (Tatchell, 1962 and Feldman-Muhsam, 1969).

During copulation, Nuttall and Merriman (1911) observed that the male O. moubata introduces all of his mouth parts into the female orifice (palps, chelicerae and hypostome). Other investigators have made similar observations on many Argasid species (Tatchell, 1967, and Feldman-Muhsam and Borut, 1971).

It has been claimed that the spermatophore in some argasid ticks is built inside the male body and that it is fully formed when extruded by the male (Nuttall and Merriman, 1911; Wagner-Yevseenko, 1968; and Tatchell, 1962).

Nuttall and Merriman (1911) also concluded that in other cases than O. moubata, the endospermatophores are introduced in toto into the (ecto)spermatophore, basing their conclusions on the findings of Christophers (1906), who found cyst-like spermatophores within the spermatheca of the females. These have probably been the endospermatophores upon their evagination in the female.

Robinson (1942), working with O. moubata, was the first to observe that the spermatophore was formed outside the male body, but erroneously believed that the capsules were squeezed into the bulb after they had been filled with the spermiophores in the ejaculatory duct, i.e. sperm remained enclosed in capsules throughout their passage from the male genital tract to the female. Feldman-Muhsam in 1967 clarified this point and gave a detailed description of the formation and anatomy of the ecto- and endospermatophore.

In Metastriata, of which A. hebraeum is a member, there are only a few records of copulation off the host; mating is usually performed on the host.

Only a few observations on the copulation of Ixodid ticks are available (Bodkin, 1918; Moorhouse, 1966; Feldman-Muhsam, 1964 ).

In Prostriata, copulation differs slightly from the Argasidae. In Ixodes the ticks mate on and very often off the host before feeding

(Graf, 1976) and not only off the hosts as in the case of the Argasid tick.

During copulation in Ixodes, the hypostome and chelicerae are inserted in the female genital aperture but the palps splay outward (Arthur, 1962; Moorhouse, 1966; and Graf, 1975).

Moorhouse (1966) claims that in Ixodes the male's chelicerae cut the tip of the spermatophore to liberate its contents. The male mouth parts in this species do not seem to play a role in transferring the ectospermatophore to the genital opening of the male, whereas the endospermatophores evaginate in the uterus in Ornithodoros ticks (Feldman-Muhsam, 1967). These evaginate in seminal receptacles in A. hebraeum.

Adult females of A. hebraeum were noticed not to fix readily to the host in the absence of males. Gladney (1971) observed similar results in A. maculatum.

Female members of Metastriata usually do not change their site of attachment until complete engorgement. Males seek the attached females and refix near them. This behaviour is reversed in Amblyomma maculatum (Gladney, 1971); and in A. hebraeum (Lounsbury, 1899).

The attraction of one sex to the other and the recognition of a potential sexual pattern suggest that chemical messengers operate between the sexes (Oliver, 1974; Graf, 1976).

In A. hebraeum mating is necessary for rapid and complete feeding, which is a prerequisite of oviposition. This appears to be characteristic of the whole Metastriata group (Gregson, 1944; Sonenshine, 1967; Pappas and Oliver, 1971; Aeschlimann and Grandjean, 1973).

In A. hebraeum, the majority of females copulate only when they are partially engorged. Some, however, may copulate after a slight feeding. Males, on the other hand, do not copulate before a blood meal. This is because spermatids are not formed until they feed. Metastriata males produce spermatids only after feeding. Similar observations have been made by Balashov (1956), Hamsher and Sonenshine (1972) and Oliver and Brinton (1972).

During the course of this study, we encountered two engorged and mated females which had failed to oviposit. This was probably due to incomplete copulation, since what may have been remnants of endospermatoophores found in their genital tracts were spermless.

It seems probable that, in A. hebraeum, a complete copulation with seminal fluid and sperm cells is necessary to induce vitellogenesis and egg-laying.

Although we cannot make any generalized conclusion based on only two observed cases, the above-mentioned possibility in A. hebraeum copulation should not be excluded.

Similar findings on O. moubata have been reported by Germond and Aeschlimann (1976). Aeschlimann (1968) successfully obtained ovipositing females of O. moubata after injecting male sexual products and living spermatids into their body cavity.

Oliver (1974) stated that although seminal fluid promotes oogenesis, it does not induce oviposition, which apparently requires the presence of conspecific spermatozoa. The author speculated on the possible role of factor(s) in the seminal fluid that stimulates initial egg development in Ornithodoros tholozani.

On the other hand, Germond and Aeschlimann (1976) report that in O. moubata the stimulus which triggers vitellogenesis and laying is not species specific and that in this tick spermioophores exert their influence on vitellogenesis and egg-laying by chemical means.

During copulation, we have observed that in A. hebraeum the male introduces only the cheliceral digits which are extruded from their shafts in the female vagina.

In three members of Metastriata, Oliver et al (1974) are uncertain whether hypostome and/or chelicerae are inserted.

The application of different parts of the male capitulum in the female genital system may be due to the anatomical structure of the female.

In A. hebraeum, the male mouth parts play no role in the transfer of the ectospermatoophore from his genital aperture to that of the female - their role is to implant the ectospermatoophore neck inside the female genital opening since throughout copulation the mouth parts do not leave

the female genital system. This view is shared by Moorhouse (1966), who worked with Ixodes holocyclus.

Some differences, however, exist between our findings and those of Ammoh-Attoh (1966), who worked on H. marginatum, a member of Metastriata.

This author claims that in Amblyomma variegatum and Hyalomma marginatum, the male uses his mouth parts to transfer, as well as to insert, the neck of his spermatophore into the female vagina. The same author mentions that multiple insemination was not observed in these ticks. In A. hebraeum, on the other hand, up to five endospermatophores were found in the seminal receptacle of one female, indicating that she had successfully been inseminated five times.

It is surprising that the results obtained by this investigator on members of Metastriata are different from those obtained by us on members of the same group and resemble in some ways those of the Argasidae and Prostriata, where the ectospermatophore is transported to the female vagina by the male mouth parts (Feldman-Muhsam et al, 1970).

Evagination of the endospermatophore in A. hebraeum is autogenous and needs neither the cutting of the ectospermatophore tip nor the pumping action exerted by the male's body on it, as believed by Nuttall and Merriman (1911). This view is also shared by Feldman-Muhsam and Borut (1971).

The endospermatophores in A. hebraeum seminal receptacles are one-lobed in contrast to the two-lobed endospermatophores of Argasid ticks.

It is puzzling that a member of Argasidae (Argas persicus) has the endospermatophore consisting of one capsule only, and that of A. reflexus consisting of the usual two for Argasids (Feldman-Muhsam, 1969).

We have observed that the evacuation of the sperm and the evagination process are autonomic, independent of any exterior interference. Feldman-Muhsam et al (1973) claim that the pressure of the gas ( $\text{CO}_2$ ) generated in the ectospermatophore causes the evagination of the endospermatophore and drives the spermiophores from the ectospermatophore into the capsules of the endospermatophore. They believe this gas

originates from enzymatic reaction at the periphery of the ectospermato-phore and produces the pressure for the evacuation of the ectospermato-phore.

Copulation in A. hebraeum, observed in this study, is more or less similar to that of Hyalomma excavatum and Ripicephalus sanguineus, as described by Feldman-Muhsam and Borut, 1971. Therefore, we conclude that in the Metastriata group, of which the three are members, copulation is, to a certain extent, similar.

During the course of this study, symbiont-like microorganisms have been observed associated with A. hebraeum. These were of two different types - one, extracellular yeast-like microorganisms, introduced in the female by the male during copulation, the other, intra-cellular and occurring naturally in the tissue organs (we observed them in the ovary, ampulla, oviducts and testis).

Extracellular symbionts in the endospermato-phores have previously been observed by Feldman-Muhsam and Havivi (1963). These form the distinct genus of Adlerocystis and live as symbionts associated with spermiophores of ticks.

In the Argasidae, these symbionts have been observed to attach to the spermiophores in most species of Ornithodoros - they do not attach in the Ixodidae (Feldman-Muhsam, 1974) nor in A. hebraeum.

It is unclear whether the intracellular microorganisms are true symbionts or Rickettsia cooneri agents with which A. hebraeum was infected. However, the finding of intracellular naturally-occurring symbiotes in the ampulla region is not uncommon. Reinhard et al (1972) have found such microorganisms in most of the organs of O. moubata.

In conclusion, we find that the presence of symbiont-like microorganisms in ticks is not uncommon (Hoogstraal, 1956; Suitor and Weiss, 1961) and the microorganisms found in the tissue organs of this tick could be true symbionts.

Tick spermatogenesis and their cytological properties have been studied by many authors (Bonnet, 1907; Samson, 1909; Casteel, 1917; Warren, 1933; Tuzet and Millot, 1937; Sharma, 1944; Balashov, 1956, 1960; Sokolov, 1958; Wagner-Jevseenko, 1958; and Goroshchenko, 1960).

More recently, different aspects of spermatogenesis have received attention from Reger (1961, 1962, 1963 and 1974), Breucker and Horstmann (1968, 1972), Khalil (1969, 1970), Oliver and Brinton (1972), Homsher and Sonenshine (1972), and Brinton et al (1974). The general pattern of spermatogenesis as described by these authors and as observed by us is to a certain extent similar in its basic pattern. However, some differences exist between the findings of these authors and ours.

There are different opinions concerning the localization of mitochondria within the sperm body. According to Casteel (1917) who worked on Argas minialus and Sokolov (1958) on Ornithodoros papillipes, mitochondria are arranged in rows and lines. This linear arrangement of mitochondria observed at the light microscopic level was recently confirmed by Breucker and Horstmann (1968) on the ultra-structural level in O. moubata. The results of our studies confirm the accumulation of mitochondria in the anterior part; however, their specific arrangement in lines or rows was never noticed in A. hebraeum. The same may be concluded from the results obtained by Opperman (1935) and Reger (1961).

The elongation process by which the spermatids of A. hebraeum develops into the mature spermatozoon is in accord with the findings of Casteel (1917); Warren (1931); Tuzet and Millot (1937); Sharma (1944); Breucker and Horstmann (1972), and Brinton et al (1974) on other ticks.

However, the method by which the inner cord gains access to the exterior at the onset of the elongation process differs from that in A. hebraeum. Contrary to the description of some authors, the inner cord does not push its way out by rupturing the outer sheath; rather it passes from a newly formed opening at its anterior end. This opening results from the detachment of a cap-like structure at a level well demonstrated by SEM. This is in accord with the findings of Borut and Feldman-Muhsam (1976) who have clearly illustrated this process in argasid and ixodid ticks.

Reger (1961) claimed that the tail end of the outer sheath is open in the spermatid of Amblyomma dissimili. This is not the case in A. hebraeum as we demonstrated by transmission and scanning microscopy.

The elongation process results in the formation of an invagination membrane termed by some authors "flagellum" (Oppermann, 1935; Tuzet and Millot, 1937; and Khalil, 1969). These authors describe an evagination process by which this flagellum protrudes outwards from the nucleate end of the spermatozoon, the nucleus migrating into its base. Wagner-Jevseenko (1958) and Diehl (personal communications) have observed in O. moubata a similar devagination of what appears to be the invagination membrane in mature sperm of A. hebraeum. In A. hebraeum, this process was never observed to occur. As no axoneme was demonstrated, we therefore refrain from calling this structure a "flagellum", and can conclude that tick sperm are aflagellate cells, motility not being produced by these structures.

The devagination of a nucleate part of the mature spermatozoon which carries the probable "acrosome" aroused some speculations on the part of the spermatozoon involved in fertilization. It is unclear whether the whole of the sperm cell penetrates the oocyte or only the devaginated part, as suggested by Tuzet and Millot (1937).

Although many authors have refrained from using this term, it has nevertheless been adopted by some authors (Wagner-Jevseenko, 1958; and Feldman-Muhsam and Havivi, 1964, among others).

As this process of devagination of the inner membrane is not universal among ticks, as has been proved by this study, it therefore cannot be definitely concluded that the sperm cell is the spermiophore.

Freeze-fractures of A. hebraeum sperm plasma membrane displays a regular arrangement of some of the particles in parallel strands. We are unable to compare these results with other tick sperm since to our knowledge the freeze-etch technique was not previously used on tick sperms. However, similar, though not identical, patterns of arrangement have been reported by Friend and Rudolf (1974) on the plasma membrane of the principal piece in guinea pig and rat sperm. These authors speculate on this phenomenon as being widespread in mammalian sperm. Our study has established the possibility that this phenomenon may also exist in invertebrate sperm.

Large peripheral granules which did not previously exist in spermatids were observed in mature spermatozoa. The significance of their presence is unclear. However, their further development and prominence in the ampulla region may suggest a role played by them in the fertilization process. This view is strengthened by their development simultaneously with the prominent development of the "acrosome".

The "pores" observed for the first time by freeze-etching on the bulbous expansions of the semisphere may be engaged in metabolic activity of this part of the spermatozoon.

In tick species, the role of the "acrosome" in the actual penetration of the egg has not been observed, nor has any enzymatic activity associated with the "acrosome" been demonstrated. Therefore, we have reservations as to the positive identification of the "acrosome" as such. Nevertheless, the structure described in this study as the "acrosome" is similar to the "acrosome" identified by Breucker and Horstmann (1968) in O. moubata and by Reger (1963) in A. dissimili.

The final location of the nucleus in the thin tail end of the sperm was observed by many investigators. Yet Sharma (1944) was the first to observe a similar localization of the "acrosome".

The differentiation of the nucleus and the "acrosome" in the early stages and young spermatozoa from Amblyomma dissimili was described by Reger (1963). However, the probable further development, formation of the acrosomal plate and its attachment to the nucleus in aging spermatozoa are not described.

This study extends the observations on the "acrosome" and demonstrates the final development of the "acrosome" and its linkage with the nucleus as it is seen in A. hebraeum. These observations are in accord with the findings of Breucker and Horstmann (1968) in O. moubata.

It may be concluded from previous papers that there is a general agreement concerning the unusual type of sperm movement. Although the club-shaped mitochondrial part is anucleate, it represents the active end with which the sperm moves forward, followed by the nucleate end.

Recent observations on living tick sperm by Reger (1974) resemble those of Christophers (1906) describing vermicular contortions of the anterior position of the sperm. Our observations concerning the movement of this part are that the tip moves in a peculiar rolling-up and folding out manner and that the bulbous expansions are in continuous motion. The general type of motility of tick spermatozoa is described by Christophers (1906) and Reger (1974) as a steady, gliding movement. Sharma (1944) describes it as a rotary movement, and Rothschild (1961) describes a sort of contractile system at the sperm surface involved in its movement.

The results of our observations on living sperm confirm both the gliding movement described by Christophers (1906), Rothschild (1961) and Reger (1974) and some rotary movement reported by Sharma (1944).

The specific function of the semisphere of the anterior end of the spermatozoon is not clearly defined. Its location at the tip of the forward moving part, its structure and the continuous motion of its complicated bulbous expansions may suggest a sort of "sensory" function, perhaps tactile.

The presence of so-called "contractile elements" (Casteel, 1917), "elastic strands" (Sokolov, 1958), "filament bundles" (Breucker and Horstmann, 1968), "subsurface filaments" (Reger, 1963) has been noticed in the spermatozoa of different ticks. They have also been observed in A. hebraeum in the dense clusters bordering the plasma membrane. As microtubules are lacking in tick sperm cells, their function may be compensated by the presence of the above-mentioned dense fibres which are noticed running along the entire length of the sperm cells. As the diameters of these fibres (100 - 150<sup>0</sup>A) correspond to the diameter of actin and myosin fibres, they may represent the above-mentioned contractile proteins.

As actin and myosin have been recently found in a variety of non-muscle cells and are thought to be involved in the various motility processes, a similar mechanism could therefore exist in the tick sperm.

Apart from the above-mentioned dense fibrillar elements, cellular processes and the fibrillar-granular region or both are probably involved in motility, as was suggested by Reger (1974).

Some freeze-etch replicas demonstrate a wavy pattern of the cellular processes. This undulation could be associated with movement as suggested by Rothschild (1961).

However, the actual mechanism and forces involved in tick movement is still not definitely clarified.

Sokolov (1958) was the first to notice the phenomenon of the entry of the sperm in the oviduct walls.

The fact that many investigators, including Balashov (1964), Khalil (1970), and ourselves, have not observed the actual process of fertilization leaves some doubt that it is performed in the usual way. Wagner-Yevseenko (1958) suggests that only the DNA enters the egg upon the disintegration of the actual sperm cells in the ampulla region.

The fact that the ampulla region does exist in A. hebraeum as in other, if not in all, tick species and that the greatest number of sperm are clustered in that region may suggest that this region is involved with the fertilization process.

Goroshchenko (1960, 1964) suggests that fertilization takes place in the ampulla regions since these contain the greatest number of sperm cells. In some way, these dissolve the ovum membrane and only the nucleus enters inside the ovum.

Balashov (1964) believes that fertilization in ixodid ticks occurs only in oocytes with a completely developed cuticle and probably after the migration of oocytes and oviducts.

Since no micropyle has been proved in the ova of ticks and since sperms are rare in the ovaries, fertilization is believed unlikely in the ovary.

The phagocytic action of the oviduct and ampulla cells on the spermatozoa has been reported by some authors (Sokolov, 1956; Wagner-Yevseenko, 1958; and Feldman-Muhsam, 1964) and was observed by us.

In the mature spermatozoon, typical endoplasmic reticulum and Golgi cisternae are not seen. However, the origin of primary lysosomes may be attributed to numerous vesicles present in the spermatozoon cytoplasm which may be morphologically changed Golgi elements. Reger (1963) demonstrated a granular cytomembrane surrounding mitochondria in *A. dissimili* which he states is endoplasmic reticulum that has undergone morphological changes during spermiogenesis.

## V. RESUME

Amblyomma hebraeum (Metastricata) est une tique relativement rare et peu étudiée. Ce travail étudie certains aspects de : la reproduction spermatogénèse, copulation, formation du spermatophore, et la maturation du sperme (celle-ci s'accomplit seulement dans la receptacle seminal de la femelle).

Nous avons utilisé la microscopie optique, la microscopie électronique à transmission, la microscopie électronique à balayage et le cryo-décapage.

L'anatomie du tractus génital femelle a été étudiée pour mieux comprendre la copulation, la localisation et le destin du sperme dans le tractus femelle. Dans le tractus génital femelle, on peut distinguer les parties suivantes :

- A. L'ovaire unique est tubulaire, qui subit un développement considérable après fécondation dans la femelle qui se nourrit sur l'hôte. Les ovocytes parviennent à maturité en grand nombre sur toute la longueur de l'ovaire et tombent dans le canal de l'ovaire. Cette période d'ovulation et d'oviposition est longue, contrairement aux Argasides qui possèdent plusieurs courtes périodes d'oviposition.
- B. Les oviductes pairs qui prennent naissance des deux côtés de l'ovaire et transportent les ovocytes vers le vagin.
- C. Une ampoule, partie élargie et à epithelium épaissi des oviductes, qui contient la réserve des spermatozoïdes mûrs, prêts à la fécondation.
- D. Le réceptacle séminal, en forme de sac, qui reçoit et conserve les endospermatophores. Ceux-ci contiennent des spermatides, qui accomplissent leur maturation, pour ensuite quitter l'endospermatophore et émigrer dans l'oviducte et l'ampoule.
- E. Le vagin qui est formé d'un cervix musculaire, nécessaire à l'expulsion des oeufs et d'un vestibulum, qui assure la communication vers l'extérieur.
- F. Une paire de glandes accessoires tubulaires.

La copulation de ce représentant des Metastricata diffère essentiellement en trois points des Argasides et des Prostricata :

- 1) A la copulation, des différentes parties du Capitulum sont introduites chez ces trois groupes systématiques dans l'orifice génitale de la femelle. Les Cheliceres chez A. hebraeum, les Cheliceres et l'hypostome chez les Prostricata et toutes les pièces buccales, les palpes incluses, chez les Argasides.

- 2) Chez A. hebraeum le mâle ne transporte pas l'ectospermatophore vers l'orifice femelle contrairement aux Argasides.
- 3) L'endospermatophore ne possède qu'un seul lobe contre deux chez les Argasides.

L'ectospermatophore, doté d'une paroi assez épaisse, transfère le sperme à la femelle par évagination d'un endospermatophore à paroi mince, où a lieu la maturation des spermatides. Cette différenciation des spermatides, est un processus tout-à-fait particulier.

Le spermatide, au moment de la copulation, est formée de deux tubes concentriques, le noyau étant placé dans la partie antérieure. Dans le réceptacle femelle, le processus d'élongation débute par l'évagination antérieure du tube intérieur et par l'invagination du tube extérieur à son extrémité postérieure. Le spermatozoïde mûr est un simple tube, de longueur double de celle de la spermatide et dont le noyau se trouve finalement dans la "queue".

La microscopie électronique à transmission, le cryodécapage et la microscopie à balayage donnent des informations nouvelles sur le processus d'élongation des spermatides. Le cylindre cytoplasmique intérieur, entouré d'un "manteau" extérieur contenant le noyau, se termine antérieurement par un hémisphère, porteur d'expansions cytoplasmiques bulboïdes. Tout le long du tube intérieur on remarque des processus cellulaires, qui continuent sur la face interne du tube extérieur. Les mitochondries, des formations denses et du cytoplasme fibrillaire et granulaire caractérisent ce stade.

Dans les spermatozoïdes mûrs, des granules périphériques apparaissent et la membrane montre son activité par l'apparition de particules hexagonales. L'"acrosome", assez spécial chez A. hebraeum, apparaît et des vésicules, pouvant être interprétées comme des lysosomes, apparaissent également.

La motilité est attribuée aux processus extérieurs et probablement au cytoplasme fibrillaire. Les spermatozoïdes se meuvent avec la partie épaisse "anucléée" vers l'avant et la queue "nucléée" vers l'arrière.

Les spermatozoïdes sont finalement "lysés" par une action enzymatique de l'épithélium ampoulaire et de l'oviducte, ainsi qu'une action autophagique des "lysosomes" des spermatozoïdes mûrs.

Deux microorganismes, probablement des symbiontes, ont été trouvés. L'un, extracellulaire (Adlerocysts), dans les endospermatophores. L'autre, intracellulaire, dans les spermatogonies et l'ovarie, l'oviducte et l'ampoule. La présence de symbiontes intracellulaires est fréquente chez les tiques, mais l'identification de cette espèce n'est pas sûre car il pourrait s'agir de Rickettsia cooneri dont A. hebraeum est porteuse.

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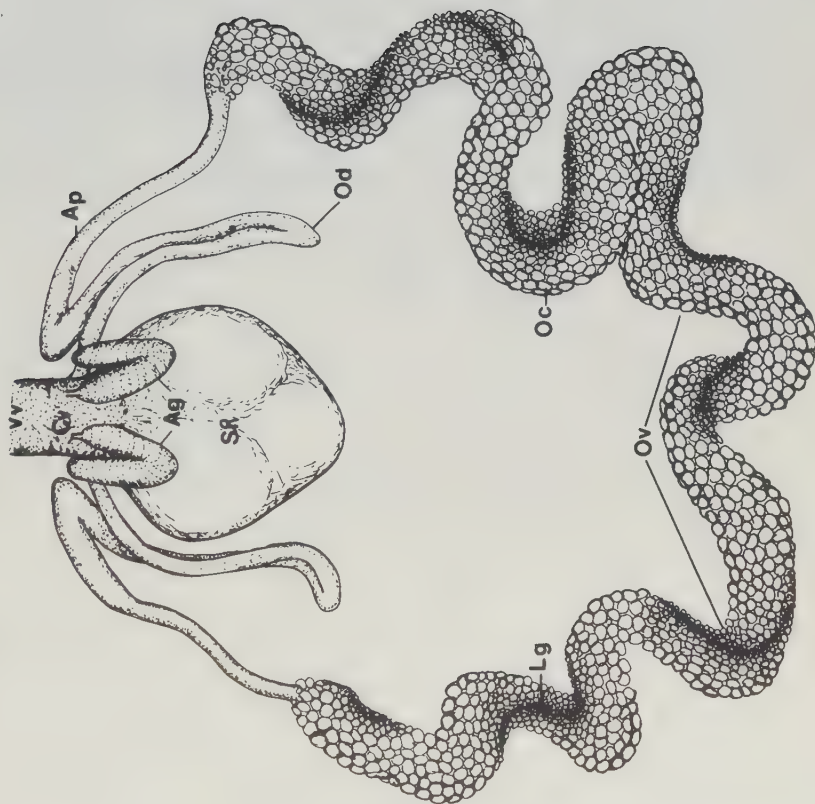
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## PLATE I

The female reproductive system composed of vestibular (Vv) and cervical vagina (Cv), leading into the seminal receptacle (SR). Oocytes (Oc) develop along the ovary (Ov), except for a sterile longitudinal groove (Lg). The ovary is connected laterally with the long paired oviducts (Od), which thicken in the regions adjacent to the vaginas forming the ampullae (Ap). Tubular accessory glands (Ag) open in the junction of both parts of the vagina.

## Female Reproductive System



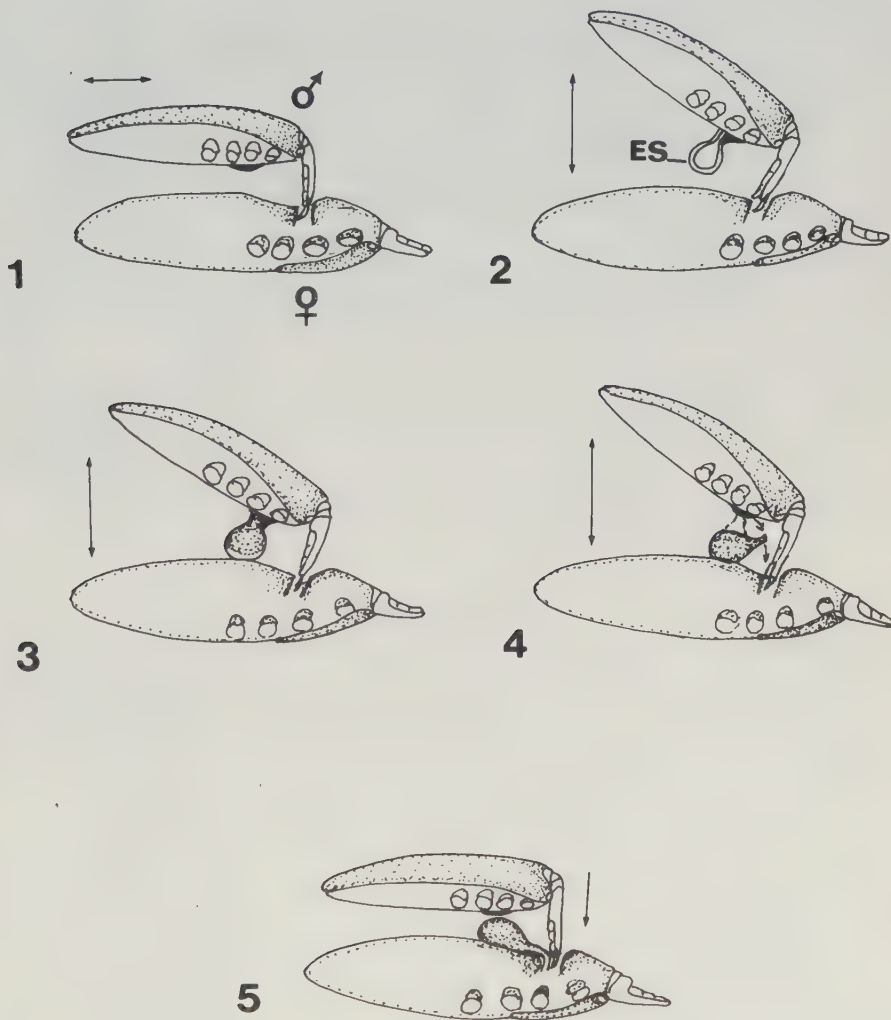
## PLATE II

Schematic diagram illustrating mode of copulation of  
Amblyomma hebraeum

- Fig. 1 : The male applies his mouth parts to the female genital opening and introduces his cheliceral digits in it.
- Fig. 2 : Ectospermatophore (ES) oozes from the male opening.
- Fig. 3 : Ectospermatophore is filled with seminal fluid, sperm and granules.
- Fig. 4 : The autonomic transfer of the neck of the ectospermatophore to the female genital opening.
- Fig. 5 : The male introduces the ectospermatophore neck into the genital opening of the female.

## Schematic diagram illustrating the mode of copulation

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### PLATE III

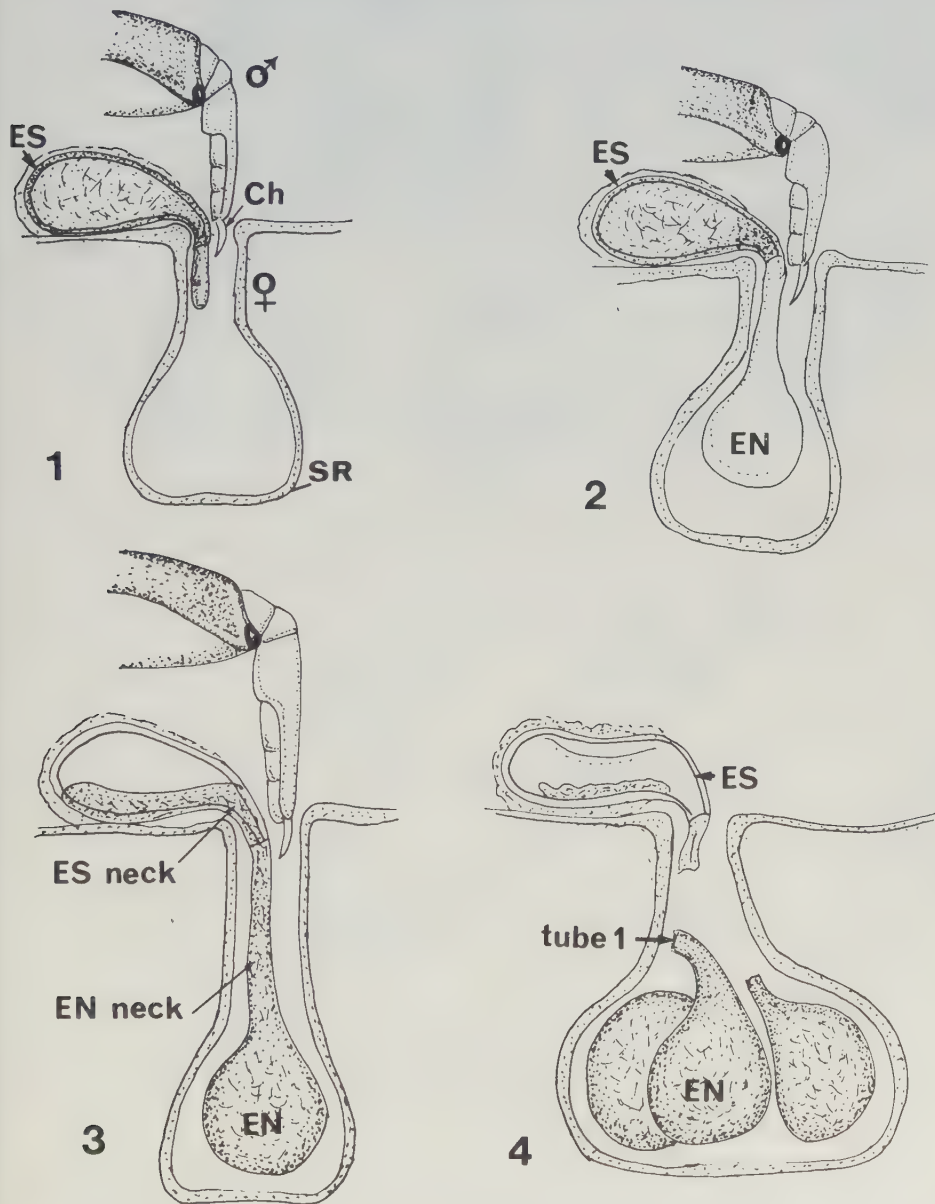
#### Evagination of the endospermatophore

Fig. 1 : The neck of the ectospermatophore is introduced in the genital opening by the male cheliceral digits. The endospermatophore begins to evaginate.

Figs. 2 Successive stages of the evagination of the endo-  
and 3 : spermatophore. The ectospermatophore contents  
are transferred to the endospermatophore.

Fig. 4 : Termination of the evagination process. The male mouth parts are removed from the female genital orifice. The two necks of the ecto- and endospermatophore separate. A part of the endospermatophore neck still attached to it represents tube 1.

Evagination of the endospermatophore



## PLATE IV

### General pattern of spermatogenesis

Fig. 1 : Spermatogenia (Sg) grouped in part of testicular cyst. N : nucleus.

Fig. 2 : Late secondary spermatocyte.

Fig. 3 : Spherical spermatid with a well developed layer of Cisternae uniformly distributed beneath the entire cell surface.

Fig. 4 : Progressive retreating of the cisternal layer from nucleus pole of the cell. Cn : Cisternae.

Fig. 5 : Beginning of spermatid elongation. Note the fusion of neighbouring cisternae forming a single cisternal cavity.

Fig. 6 : Further elongation of the spermatid and the cisternal cavity. Cp : cellular processes.

Fig. 7 : Evagination of a cytoplasmic protuberance into the cisternal cavity carrying at its tip small bulbous expansions (be), the last row of which extend backwards along the protuberance length.

m : mitochondria

Fig. 8 : The cytoplasmic protuberance continues forming an inner cord (ic) which is surrounded by an outer sheath (os). Simultaneously with inner cord elongation the spermatid nucleus (N) migrates within the outer sheath to the opposite end of the cell.

Fig. 9 : The last stage of spermatid elongation within the male. In this stage spermatids are transferred to the female where further development occurs.

be : bulbous expansions

ic : inner cord

N : Nucleus

os : outer sheath

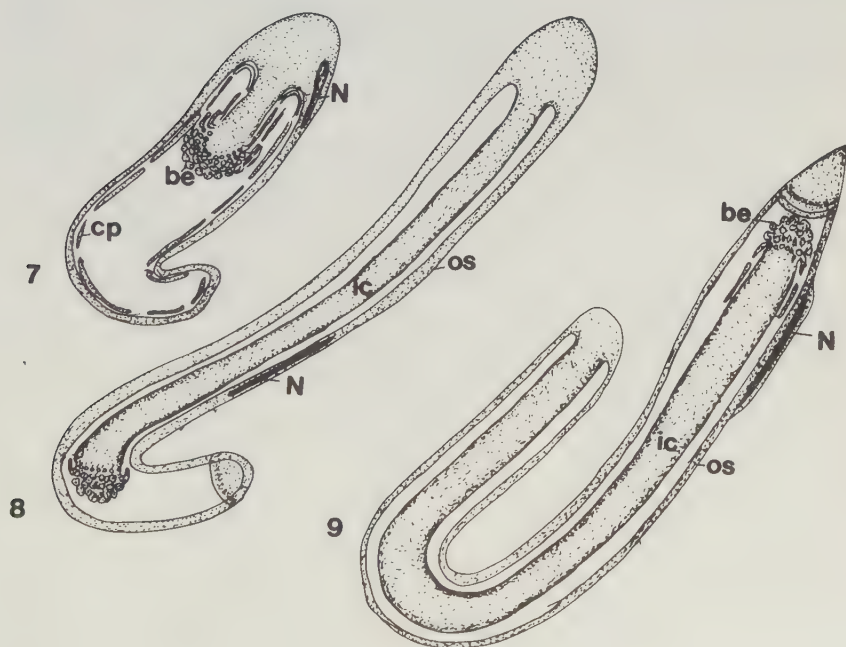
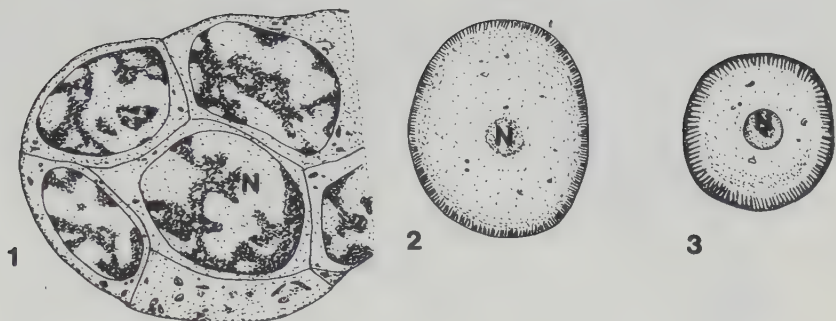
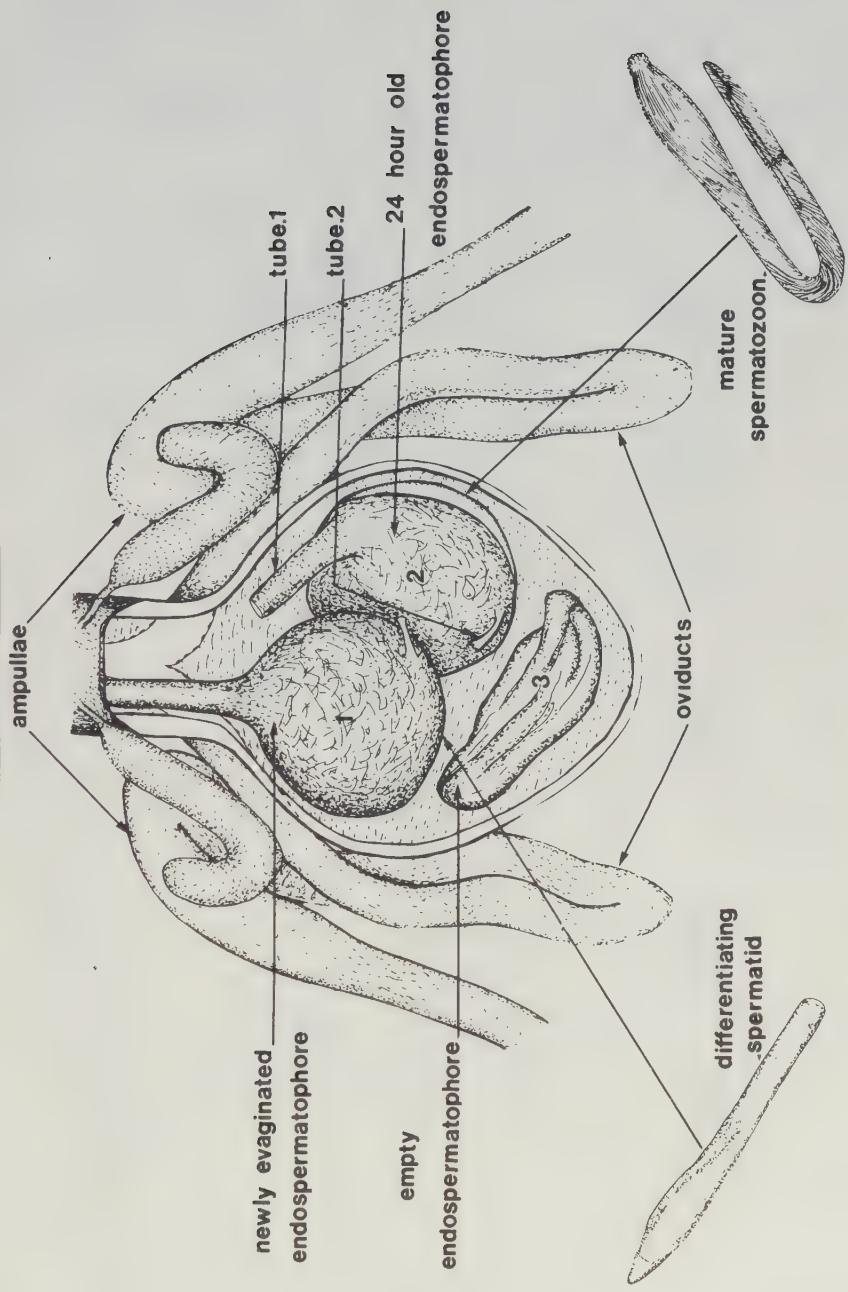


PLATE V

Detailed schematic diagram of the seminal receptacle  
and enclosed endospermatothecae

seminal receptacle



## PLATE VI

### Nucleo-acrosomal region of the spermatozoon

(Arrows indicate its localization within the mature sperm)

The acrosomal plate (AP) and the nucleus (N) are connected together at the nuclear base by a long rod (R) centrally placed in the nucleus.

The invagination membrane (IM) lines the acrosomal cavity (AC) in which numerous tubular vesicles (tv) are situated.

AC : acrosomal canal  
AP : acrosomal plate  
cp : cellular processes  
IM : invagination membrane  
m : mitochondria  
N : Nucleus  
N base : nuclear base  
PG : peripheral granules  
R : rod  
tv : tubular vesicles

# NUCLEO - ACROSOMAL REGION OF SPERMATOZOON

